Elements of Bioinformatics Autumn 2011

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http://www.cs.helsinki.fi/courses/582606/2011/s/k/1

Lecture Mon 31.10.

GENE PREDICTION

Molecular biology concepts recap

Nucleotides A, C, G, T

gene

DNA

...TACCTACATCCACTCATC...AGCTACGTTCCCCGACTACGACATGGTGATT

5’ ...ATGGATGTAGGTGAGTAG...TCGATGCAAGGGGCTGATGCTGTACCACTAA... 3’

exon intron exon

transcription regulation

RNA

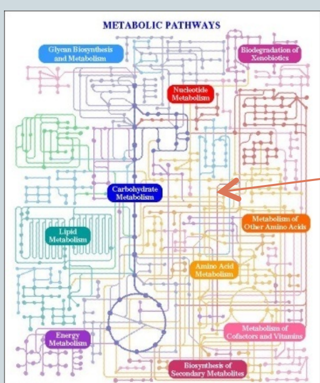
codon

...AUGGAUGUAGAUGGGCUGAUGCUGUACCACUAA

translation

Protein

MDVDGLMLYH regulation



entsyme

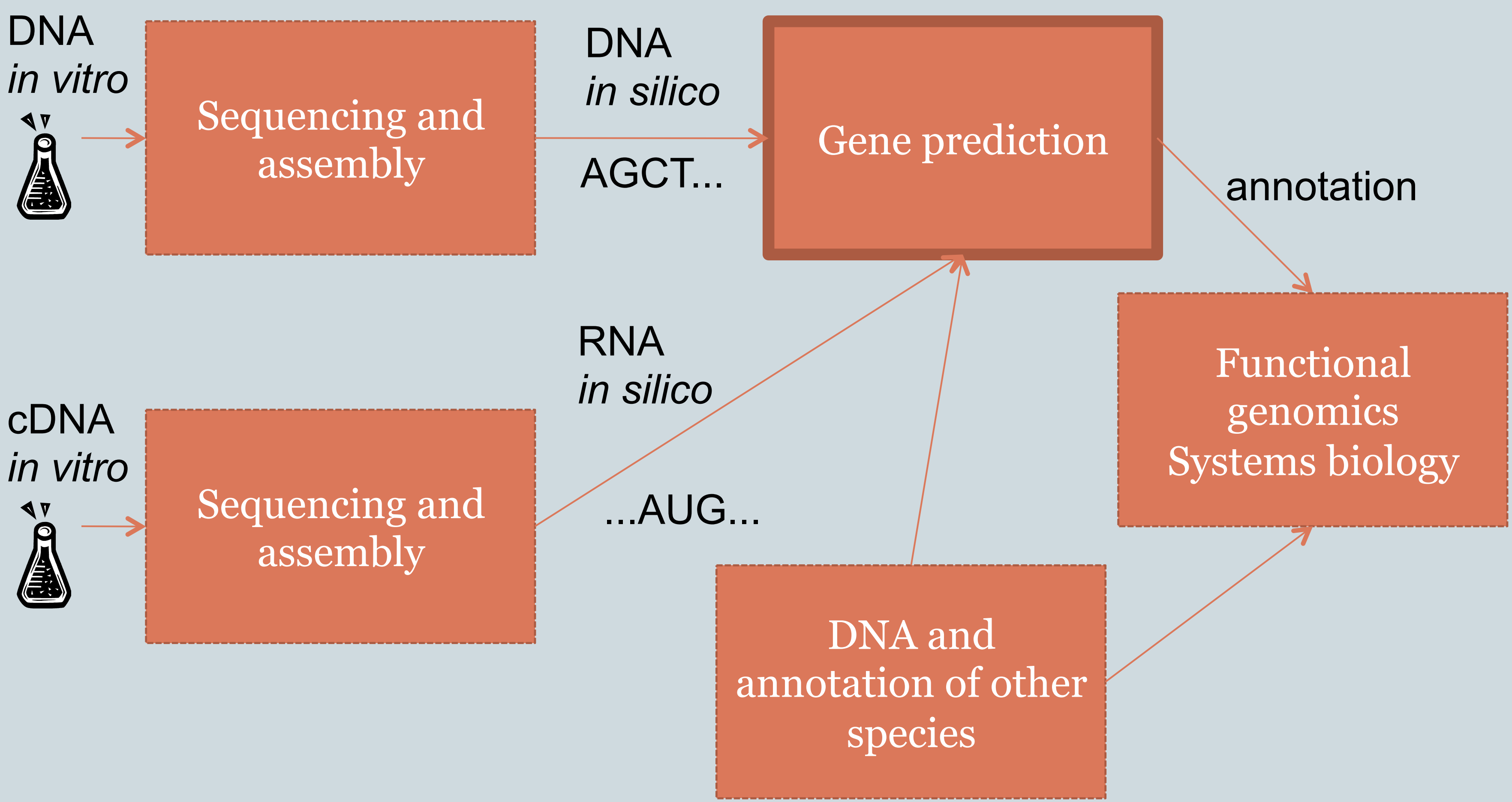
recombination

Mother DNA C G A A

Father DNA T C C T

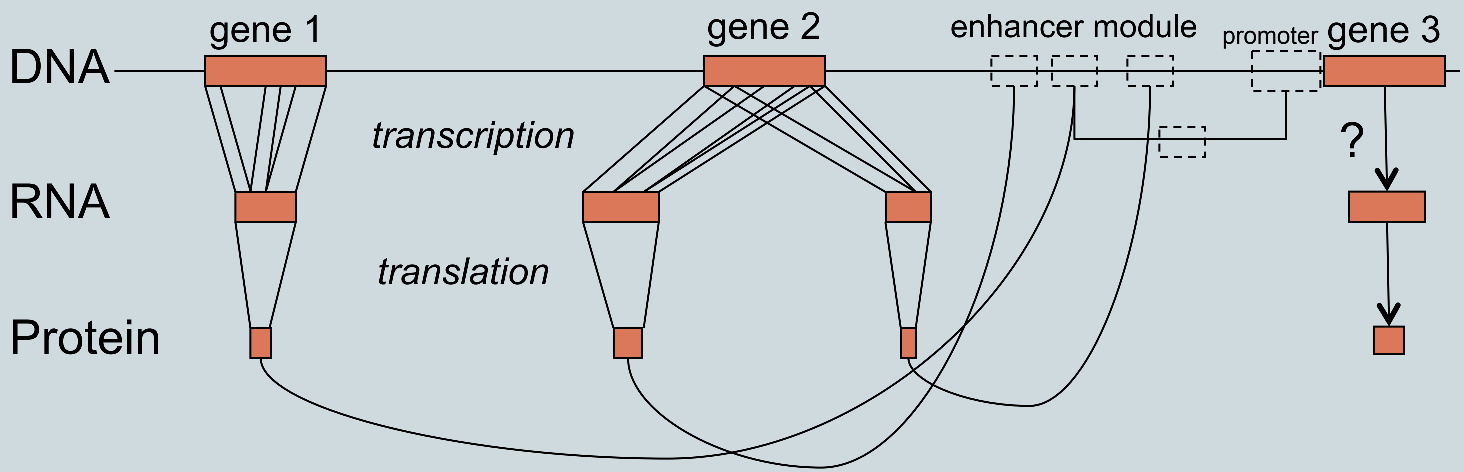
Daughter DNA C C C A

Genome analysis pipeline



Gene structure

- Genes
  - start and stop codons
  - exons, introns (in eukaryotic organisms)
- Promoter regions
  - binding sites for regulatory proteins



Typical eukaryotic gene

- ATG –start codon, TAA –stop codon
- yellow: exons, blue: introns, red: untranslated region
- black: upstream (promoter) and downstream regions

http://en.wikipedia.org/wiki/File:AMY1gene.png

Gene prediction: main approaches

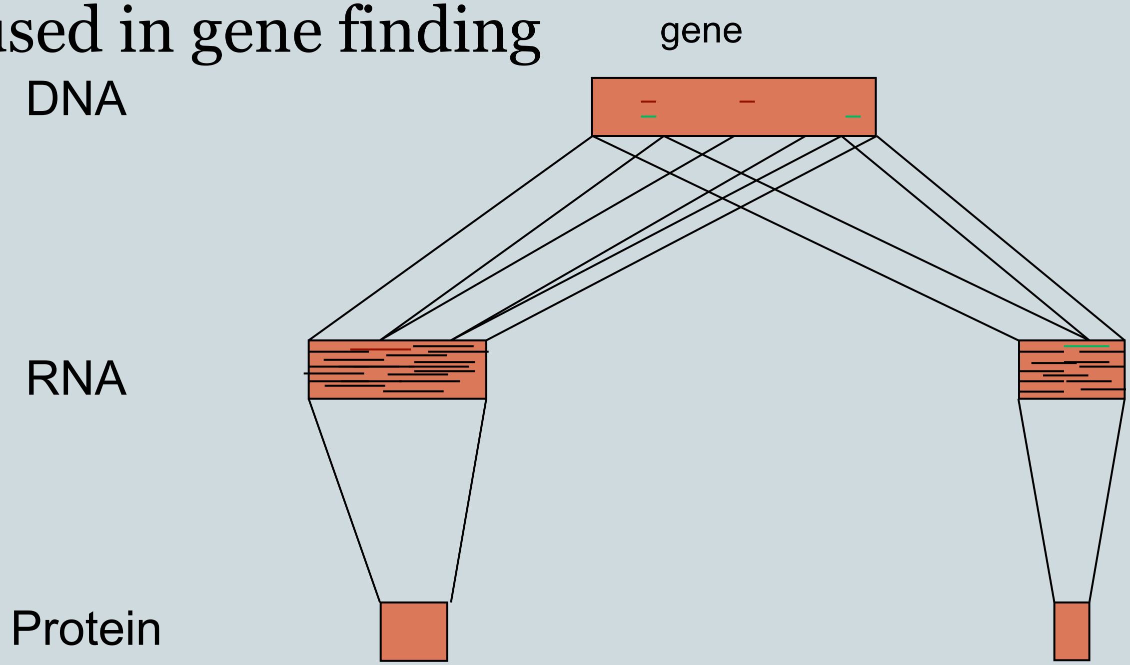
- Evidence-based gene finding: identify mRNA sequences expressed by the organism and map them back to the genome
- Ab initio gene prediction: detecting the 'signal' of functional elements via statistical approaches or matching against a database of known motifs
- Comparative genomics approaches: detect conserved DNA regions by comparing a large set of related genomes

Evidence-based gene finding

- In evidence-based gene finding, one assumes that there is access to mRNA or protein sequences expressed by the organism
  - RNA-seq is one suitable experimental technique for mRNA
  - Peptide sequencing via tandem mass spectrometry gives amino acid sequences
- Target genome is searched for sequences that match the expressed mRNA or protein sequences
  - Sequence alignment problem using, e.g. BLAST
  - For prokaryotic genes, reltively straight-forwards
  - Exon-intron structure of eukaryotic genes is a complication

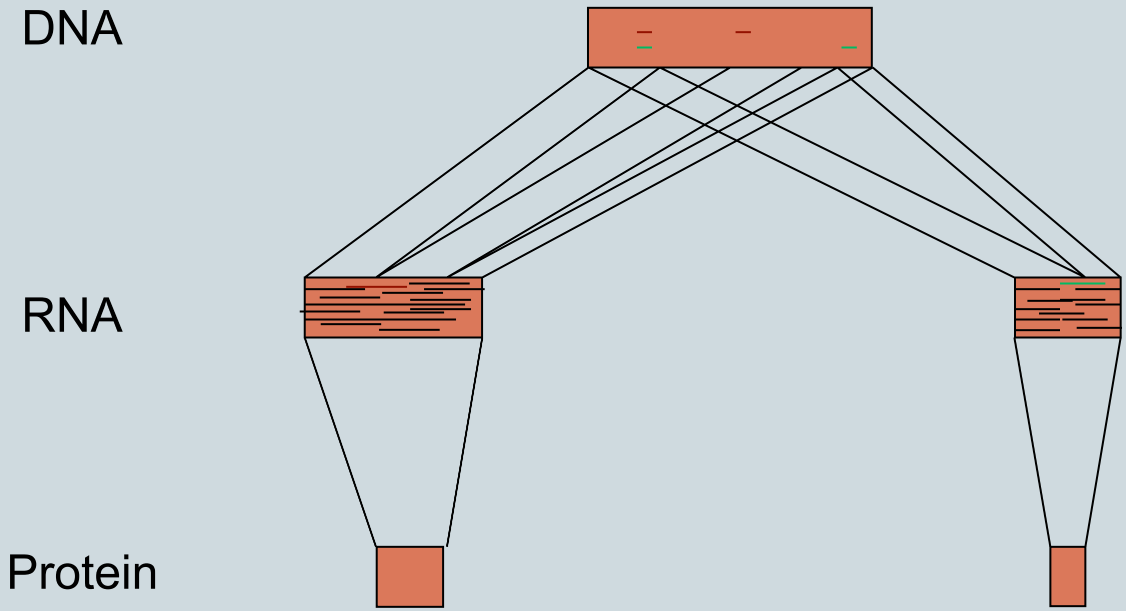
RNA-seq for Gene finding

- RNA-seq is a short-read sequencing technology to measure the transcriptome, i.e. all expressed mRNA in a given sample
- Mainly used to study the function of genes, but can also be used in gene finding



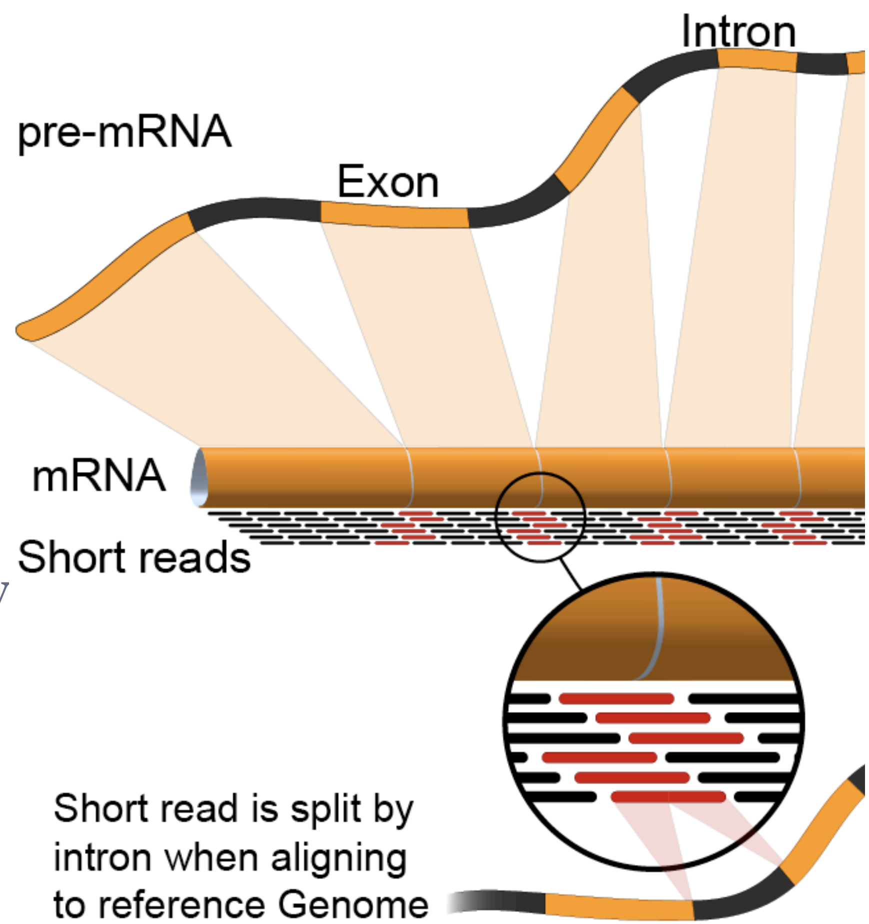
RNA-seq for gene finding

- Two alternative approaches:
  - Assemble mRNA from short reads and match the mRNA transcript to the genome (taking introns into account)
  - Align the short reads of cDNA directly to the genome and vote for exons.



Eukaryotic Gene finding with RNA-seq data

- Consider the first approach: matching the RNA-seq reads to the target genome
- Some reads may cross exon-exon boundary
  - In the target genome these read sequence will be split by an intron
  - Generally we may not know where the exon-exon boundaries are
- Exercises: discover solutions to this problem



Eukaryotic Gene finding with known protein sequences

- Consider matching known protein sequence to the target genome
- As only exons are translated, when matching the protein sequence into the target genome, one needs to consider where the introns might be located
- With dynamic programming one compute a score matrix S, so that S[i,j,k] gives the maximum score of exons in dna[1…j] translating into protein[1…i] with k introns
- Exercises: details of the method

Gene finding example

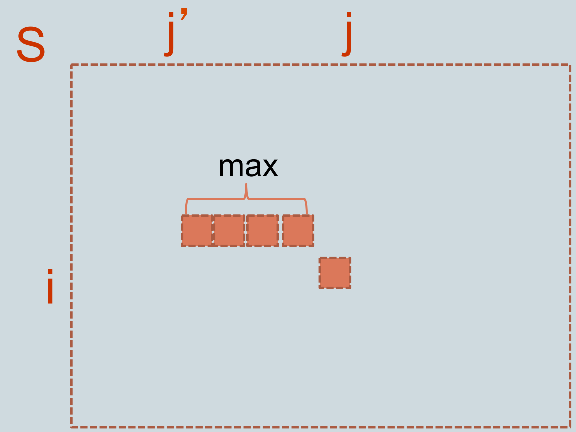
j

...ATGACATATGTAAATGGTAGCTACGTACGATCGAGGTAGCAGGCCTATTAA...

MTYVNGSYAY

i

match score = 1
mismatch score = 0
indel score = -∞



S[i,j,0]=-∞
S[i,j,1]=10

(exercise develops the recursion)

...ATGACATATGTAAATGGTAGCTACgtacgatcgaggtagcagGCCTATTAA...

M T Y V N G S Y A Y

sliding window maxima technique:

$\underbrace{3,5,6,2,3,4,}_{\max = 6}\ 7 \rightarrow 3,\underbrace{5,6,2,3,4,\ 7}_{\max = 7}$

Enough to store right-most maxima: (i,A[i]) such that there is no A[j]>A[i], for indices i<j inside the sliding window.

Evidence-based gene finding

- Major limitations of evidence-based approach is coverage
  - mRNA approach:
    - Not all genes are expressed all the time or all tissues, so mRNA will not in general cover the all genes
  - Known protein approach:
    - Not all proteins have been sequenced, corresponding genes would be missed
    - What if the target genome contains previously unknown genes?
- For larger coverage,need ab initio tools that do not require observing the gene products

Ab initio gene prediction

- What can be deduced from just the genome content?
- Introns often have markers at both ends (gt…ag,…), but these markers also appear in other places.
- Statistical properties need to be used to distinguish between coding and non-coding regions.
- Already non-trivial for prokaryotes as not all start codon – stop codon pairs (*open reading frames*, ORFs) correspond to genes.
- Hidden Markov Model (HMM) –techniques can be used for this prediction task. (next lecture)

Biological words: k-mer statistics

- To understand statistical approaches to gene prediction, we need to study what is known about the structure and statistics of DNA.
  - 1-mers: individual nucleotides (bases)
  - 2-mers: dinucleotides (AA, AC, AG, AT, CA, ...)
  - 3-mers: codons (AAA, AAC, ...)
  - 4-mers and beyond

1-mers: base composition

- Typically DNA exists as *duplex* molecule (two complementary strands)

```
5'-GGATCGAAGCTAAGGGCT-3'
3'-CCTAGCTTCGATTCCCGA-5'
```

Top strand:	7 G, 3 C, 5 A, 3 T
Bottom strand:	3 G, 7 C, 3 A, 5 T
Duplex molecule:	10 G, 10 C, 8 A, 8 T
Base frequencies:	10/36 10/36 8/36 8/36

These are something we can determine experimentally.

fr(G + C) = 20/36, fr(A + T) = 1 – fr(G + C) = 16/36

G+C content

- fr(G + C), or *G+C content* is a simple statistics for describing genomes
- Notice that one value is enough to characterise fr(A), fr(C), fr(G) and fr(T) for duplex DNA
- Is G+C content (= base composition) able to tell the difference between genomes of different organisms?
- Is G+C content able to tell the difference between coding and non-coding regions?

G+C content and genome sizes (in megabasepairs, Mb) for various organisms

Mycoplasma genitalium	31.6%	0.585
Escherichia coli K-12	50.7%	4.693
Pseudomonas aeruginosa PAO1	66.4%	6.264
Pyrococcus abyssi	44.6%	1.765
Thermoplasma volcanium	39.9%	1.585
Caenorhabditis elegans	36%	97
Arabidopsis thaliana	35%	125
Homo sapiens	41%	3080

Base frequencies in duplex molecules

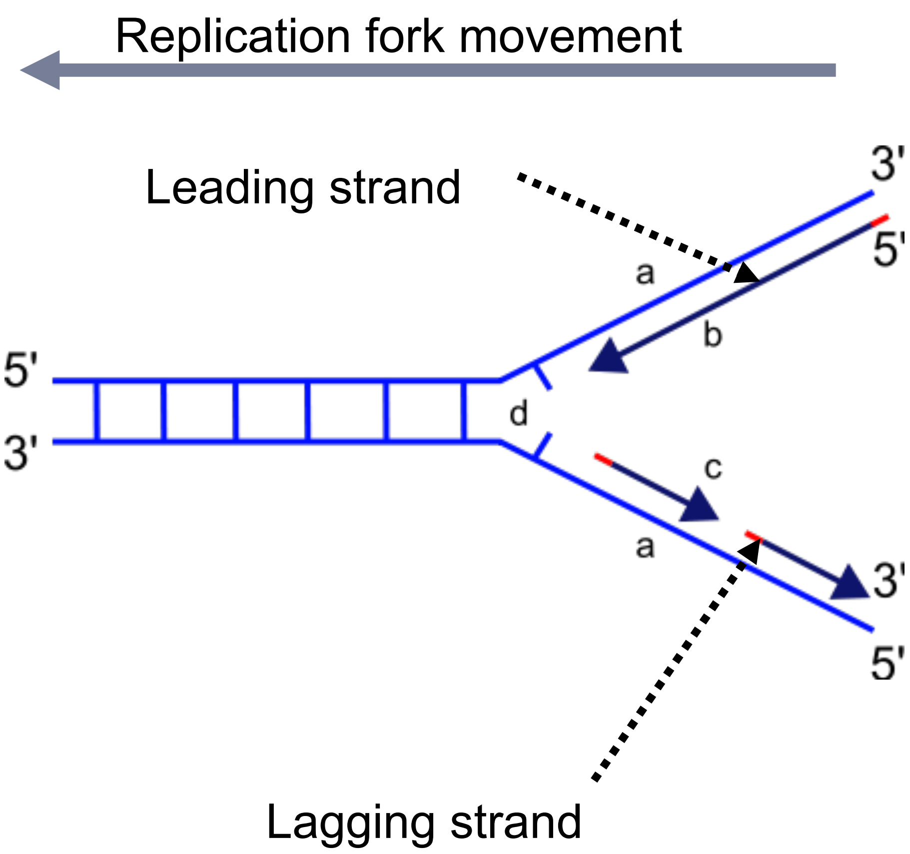
- Consider a DNA sequence generated randomly, with probability of each letter being independent of position in sequence
- You could expect to find a uniform distribution of bases in genomes…

```
5’-...GGATCGAAGCTAAGGGCT...-3’
3’-...CCTAGCTTCGATTCCCGA...-5’
```

- This is not, however, the case in genomes, especially in prokaryotes
  - This phenomena is called *GC skew*

DNA replication fork

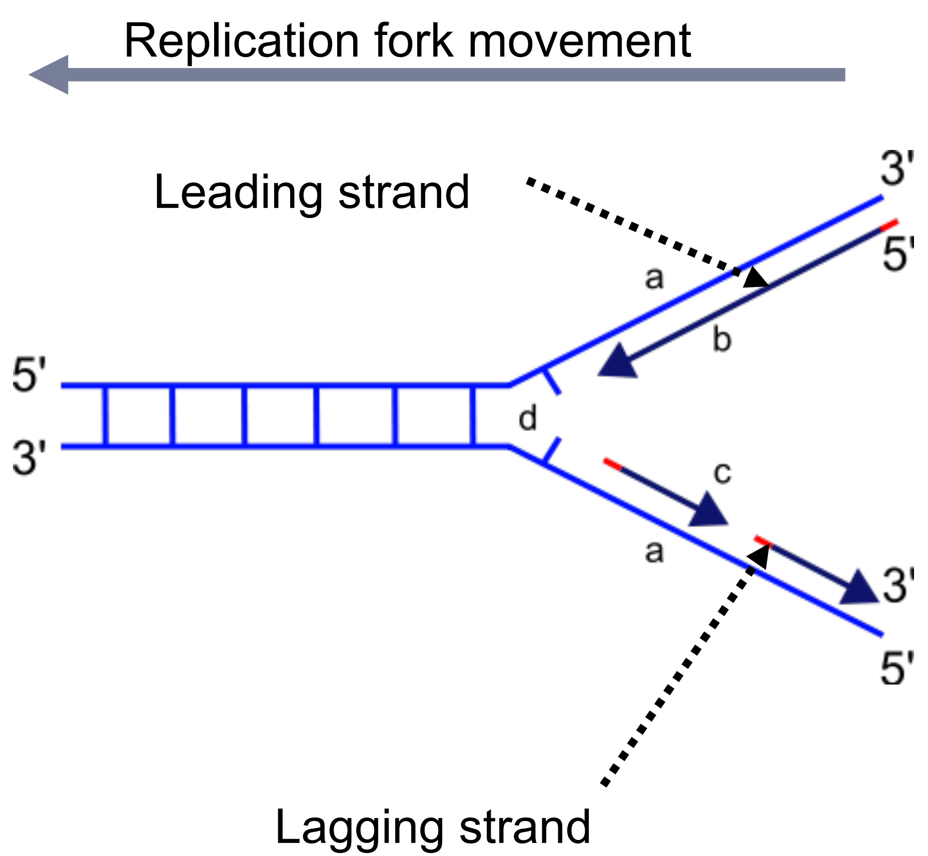
- When DNA is replicated, the molecule takes the *replication fork* form
- New complementary DNA is synthesised at both strands of the ”fork”
- New strand in 5’ -3’ direction corresponding to replication fork movement is called *leading strand* and the other *lagging strand*



Replication fork

DNA replication fork

- This process has specific starting points in genome (*origins of replication)*
- Observation: Leading strands have an excess of G over C
- This can be described by *GC skew* statistics



Replication fork

GC skew

- GC skew is defined as (#G - #C) / (#G + #C)
- It is calculated at successive positions in intervals (windows) of specific width

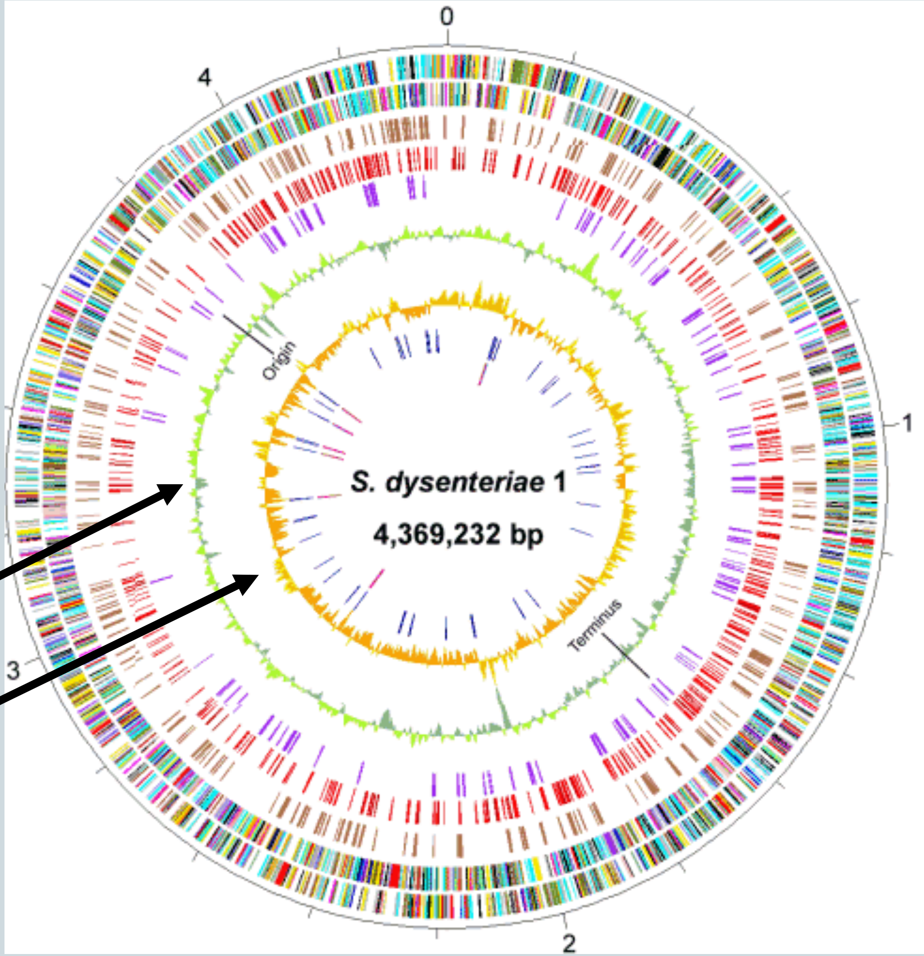
```
5’-...GGATCGAAGCTAAGGGCT...-3’
3’-...CCTAGCTTCGATTCCCGA...-5’
```

(4 – 2) / (4 + 2) = 1/3

(3 – 2) / (3 + 2) = 1/5

G-C content & GC skew

- G-C content & GC skew statistics can be displayed with a *circular genome map*



G+C content

GC skew

(10kb window size)

Chromosome map of *S. dysenteriae*, the nine rings describe different properties of the genome http://www.mgc.ac.cn/ShiBASE/circular_Sd197.htm

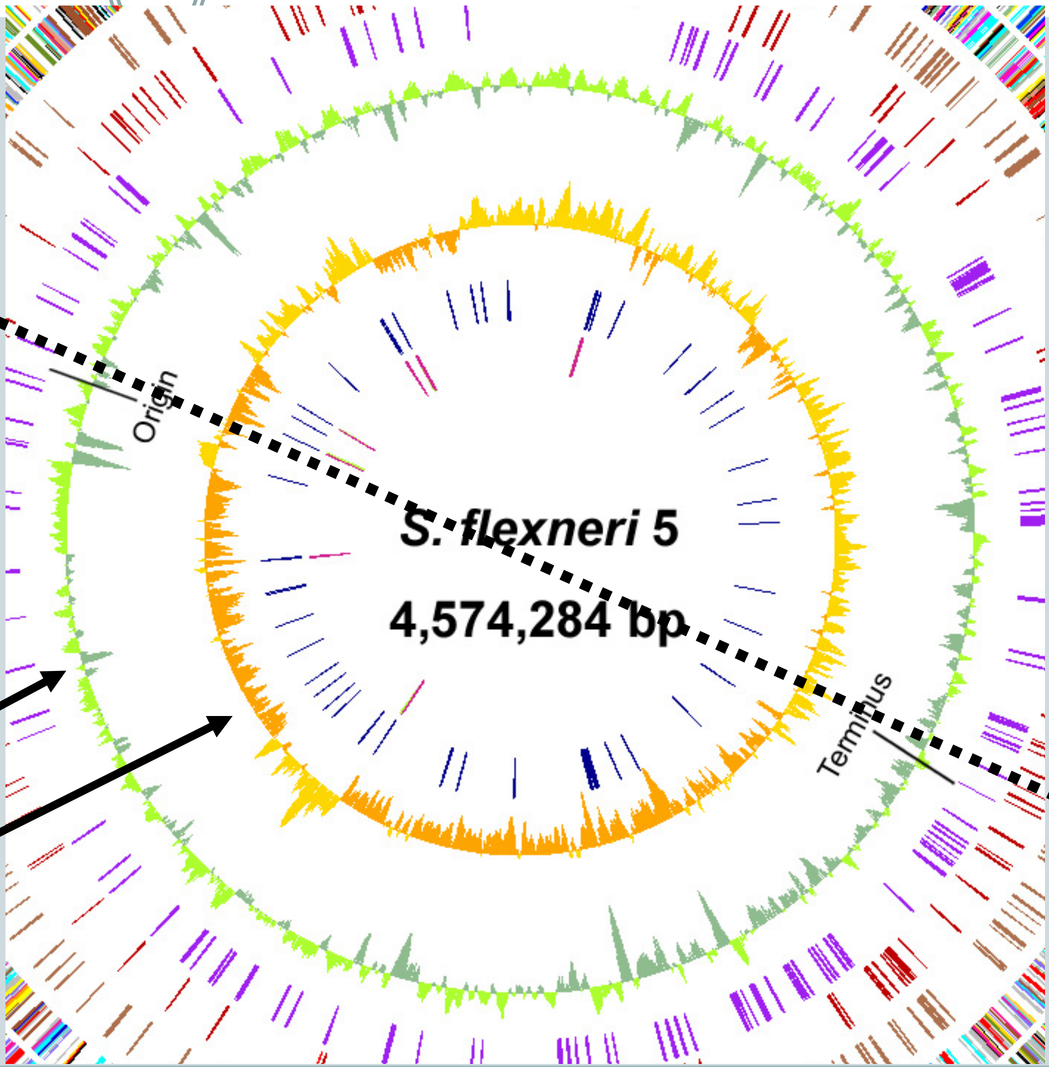
GC skew

- GC skew often changes sign at origins and termini of replication

G+C content

GC skew

(10kb window size)



Nie et al., BMC Genomics, 2006

i.i.d. model for nucleotides

- Assume that bases
  - occur **i**ndependently of each other
  - bases at each position are **i**dentically **d**istributed
- Probability of the base A, C, G, T occuring is p_A, p_C, p_G, p_T, respectively
  - For example, we could use $p_A=p_C=p_G=p_T=0.25$ or estimate the values from known genome data
- Joint probability is then just the product of independent variables
  - For example, $P(TG) = p_T \, p_G$

Refining the i.i.d. model

- i.i.d. model describes some organisms well but fails to characterise many others

- We can refine the model by having the DNA letter at some position depend on letters at preceding positions

…TCGTGACGCCG ?

Sequence context to consider

First-order Markov chains

X_t

…TCGTGACGCCG ?

X_{t-1}

- Let' s assume that in sequence X the letter at position t, X_t, depends only on the previous letter X_{t-1} (*first-order markov chain*)
- Probability of letter b occuring at position t given X_{t-1} = a: $p_{ab} = P(X_t = b \mid X_{t-1} = a)$
- We consider *homogeneous* markov chains: probability p_{ab} is independent of position t

Estimating p_{ab}

- We can estimate probabilities p_{ab} (”the probability that b follows a”) from observed dinucleotide frequencies

	A	C	G	T
A	p_{AA}	p_{AC}	p_{AG}	p_{AT}
C	p_{CA} +	p_{CC} +	p_{CG} +	p_{CT}
G	p_{GA}	p_{GC}	p_{GG}	p_{GT}
T	p_{TA}	p_{TC}	p_{TG}	p_{TT}

Frequency of dinucleotide AT in sequence

Base frequency fr(C)

…the values p_{AA}, p_{AC}, …, p_{TG}, p_{TT} sum to 1

Estimating p_{ab}

- $p_{ab} = P(X_t = b \mid X_{t-1} = a) = \dfrac{P(X_t = b, X_{t-1} = a)}{P(X_{t-1} = a)}$

Probability of transition a -> b

Dinucleotide frequency

Base frequency of nucleotide a, fr(a)

0.052 / 0.345 ≈ 0.151

	A	C	G	T
A	0.146	0.052	0.058	0.089
C	0.063	0.029	0.010	0.056
G	0.050	0.030	0.028	0.051
T	0.086	0.047	0.063	0.140

$P(X_t = b, X_{t-1} = a)$

	A	C	G	T
A	0.423	0.151	0.168	0.258
C	0.399	0.184	0.063	0.354
G	0.314	0.189	0.176	0.321
T	0.258	0.138	0.187	0.415

$P(X_t = b \mid X_{t-1} = a)$

Simulating a DNA sequence

- From a transition matrix, it is easy to generate a DNA sequence of length n:
  - First, choose the starting base randomly according to the base frequency distribution
  - Then, choose next base according to the distribution $P(x_t \mid x_{t-1})$ until n bases have been chosen

T T C T T C A A

	A	C	G	T
A	0.423	0.151	0.168	0.258
C	0.399	0.184	0.063	0.354
G	0.314	0.189	0.176	0.321
T	0.258	0.138	0.187	0.415

$P(X_t = b \mid X_{t-1} = a)$

Example Python code for generating DNA sequences with first-order Markov chains.

```
#!/usr/bin/env python

import sys, random

n = int(sys.argv[1])
```

Initialisation: use packages ’sys’ and ’random’, read sequence length from input.

```
tm = {'a' : {'a' : 0.423, 'c' : 0.151, 'g' : 0.168, 't' : 0.258},
      'c' : {'a' : 0.399, 'c' : 0.184, 'g' : 0.063, 't' : 0.354},
      'g' : {'a' : 0.314, 'c' : 0.189, 'g' : 0.176, 't' : 0.321},
      't' : {'a' : 0.258, 'c' : 0.138, 'g' : 0.187, 't' : 0.415}}

pi = {'a' : 0.345, 'c' : 0.158, 'g' : 0.159, 't' : 0.337}
```

Transition matrix tm and initial distribution pi.

```
def choose(dist):
    r = random.random()
    sum = 0.0
    keys = dist.keys()
    for k in keys:
        sum += dist[k]
        if sum > r:
            return k
    return keys[-1]
```

Function choose(), returns a key (here ’a’, ’c’, ’g’ or ’t’) of the dictionary ’dist’ chosen randomly according to probabilities in dictionary values.

```
c = choose(pi)
for i in range(n - 1):
    sys.stdout.write(c)
    c = choose(tm[c])
sys.stdout.write(c)
sys.stdout.write("\n")
```

Choose the first letter, then choose next letter according to $P(x_t \mid x_{t-1})$.

Simulating a DNA sequence

- Now we can quickly generate sequences of arbitrary length…

```
ttcttcaaaataaggatagtgattcttattggcttaagggataacaatttagatctttttcatgaatcatgtatgtcaacgttaaaagttgaactgcaataagttc
ttacacacgattgtttatctgcgtgcgaagcatttcactacatttgccgatgcagccaaaagtatttaacatttggtaaacaaattgacttaaatcgcgcacttaga
gtttgacgtttcatagttgatgcgtgtctaacaattactttagtttttaaatgcgtttgtctacaatcattaatcagctctggaaaaacattaatgcatttaaac
cacaatggataattagttacttattttaaaattcacaaagtaattattcgaatagtgccctaagagagtactggggttaatggcaaagaaaattactgtagtgaaga
ttaagcctgttattatcacctgggtactctggtgaatgcacataagcaaatgctacttcagtgtcaaagcaaaaaaatttactgataggactaaaaccctttattt
ttagaatttgtaaaaatgtgacctcttgcttataacatcatatttattgggtcgttctaggacactgtgattgccttctaactcttatttagcaaaaaattgtcata
gctttgaggtcagacaaacaagtgaatggaagacagaaaaagctcagcctagaattagcatgttttgagtggggaattacttggttaactaaagtgttcatgactgt
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ggataagaaaacagcaaacaaatttagtattattttcctagtaaaaagcaaacatcaaggagaaattggaagctgcttgttcagtttgcattaaattaaaaatttat
ttgaagtattcgagcaatgttgacagtctgcgttcttcaaataagcagcaaatcccctcaaaattgggcaaaaacctaccctggcttctttttaaaaaaccaagaaa
agtcctatataagcaacaaatttcaaacctttttgttaaaaattctgctgctgaataaataggcattacagcaatgcaattaggtgcaaaaaaggccatcctctttct
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caatgtaatcatattctagagtattaaaaatacaaaaagtacaattagttatgcccattggcctggcaatttatttactccactttccacgttttggggatattta
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atataaactgatttaagtgtatttgctaataacattttcgaaaaggaatattatggtaagaattcataaaaatgtttaatactgatacaactttcttttatatcctc
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acctggtataaaaacatcaagtaacattcaaattgcaaatgaaaactaaccgatctaagcattgattgatttttctcatgcctttcgcctagttttaataaacgcgc
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atgacattacaatttttctatgttttgttctgttgagtcaaagtgcatgcctacaattctttcttatatagaactagacaaaatagaaaaaggcacttttggagtct
gaatgtcccttagtttcaaaaaggaaattgttgaatttttttgtggttagttaaattttgaacaaactagtatagtggtgacaaacgatcaccttgagtcggtgacta
taaaagaaaaaggagattaaaaatacctgcggtgccacatttttttgttacgggcatttaaggtttgcatgtgttgagcaattgaaacctacaactcaataagtcatg
ttaagtcacttctttgaaaaaaaaaaaagaccctttaagcaagctc
```

Simulating a DNA sequence

Dinucleotide frequencies

	Simulated	Observed
aa	0.145	0.146
ac	0.050	0.052
ag	0.055	0.058
at	0.092	0.089
ca	0.065	0.063
cc	0.028	0.029
cg	0.011	0.010
ct	0.058	0.056
ga	0.048	0.050
gc	0.032	0.030
gg	0.029	0.028
gt	0.050	0.051
ta	0.084	0.086
tc	0.052	0.047
tg	0.064	0.063
tt	0.138	0.0140

$n = 10000$

Simulating a DNA sequence

- The model is able to generate correct proportions of 1- and 2-mers in genomes...
- ...but fails with k=3 and beyond.

```
ttcttcaaaataaggatagtgattcttattggcttaagggataacaatttagatctttttcatgaatcatgtatgtcaacgttaaaagttgaactgcaataagttc
ttacacacgattgtttatctgcgtgcgaagcatttcactacatttgccgatgcagccaaaagtatttaacatttggtaaacaaattgacttaaatcgcgcacttaga
gtttgacgtttcatagttgatgcgtgtctaacaattactttagtttttaaatgcgtttgtctacaatcattaatcagctctggaaaaacattaatgcatttaaac
cacaatggataattagttacttattttaaaattcacaaagtaattattcgaatagtgccctaagagagtactggggttaatggcaaagaaaattactgtagtgaaga
ttaagcctgttattatcacctgggtactctggtgaatgcacataagcaaatgctacttcagtgtcaaagcaaaaaaatttactgataggactaaaaccctttattt
ttagaatttgtaaaaatgtgacctcttgcttataacatcatatttattgggtcgttctaggacactgtgattgccttctaactcttatttagcaaaaaattgtcata
gctttgaggtcagacaaacaagtgaatggaagacagaaaaagctcagcctagaattagcatgttttgagtggggaattacttggttaactaaagtgttcatgactgt
tcagcatatgattgttggtgagcactacaaagatagaagagttaaactaggtagtggtgatttcgctaacacagttttcatacaagttctattttctcaatggtttt
ggataagaaaacagcaaacaaatttagtattattttcctagtaaaaagcaaacatcaaggagaaattggaagctgcttgttcagtttgcattaaattaaaatttat
ttgaagtattcgagcaatgttgacagtctgcgttcttcaaataagcagcaaatcccctcaaaattgggcaaaaacctaccctggcttctttttaaaaaaccaagaaa
agtcctatataagcaacaaatttcaaacctttgttaaaaattctgctgctgaataaataggcattacagcaatgcaattaggtgcaaaaaaggccatcctctttct
tttttgtacaattgttcaagcaactttgaatttgcagatttaacccactgtctatatgggacttcgaattaaattgactggtctgcatcacaaatttcaactgcc
caatgtaatcatattctagagtattaaaaatacaaaaagtacaattagttatgcccattggcctggcaatttatttactccactttccacgttttggggatattta
acttgaatagttcacaatcaaaacataggaaggatctactgctaaaagcaaaagcgtattggaatgataaaaaactttgatgtttaaaaaactacaaccttaatgaa
ttaaagttgaaaaaatattcaaaaaaagaaattcagttcttggcgagtaatatttttgatgtttgagatcagggttacaaaataagtgcatgagattaactcttcaa
atataaactgatttaagtgtatttgctaataacattttcgaaaaggaatattatggtaagaattcataaaaatgtttaatactgatacaactttcttttatatcctc
catttggccagaatactgttgcacacaactaattggaaaaaaaatagaacgggtcaatctcagtgggaggagaagaaaaaagttggtgcaggaaatagtttctacta
acctggtataaaaacatcaagtaacattcaaattgcaaatgaaaactaaccgatctaagcattgattgatttttctcatgcctttcgcctagttttaataaacgcgc
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atgacattacaattttctatgttttgttctgttgagtcaaagtgcatgcctacaattctttcttatatagaactagacaaaatagaaaaaggcacttttggagtct
gaatgtcccttagtttcaaaaaggaaattgttgaatttttttgtggttagttaaattttgaacaaactagtatagtggtgacaaacgatcaccttgagtcggtgacta
taaaagaaaaaggagattaaaaatacctgcggtgccacatttttttgttacgggcatttaaggtttgcatgtgttgagcaattgaaacctacaactcaataagtcatg
ttaagtcacttctttgaaaaaaaaaaagaccctttaagcaagctc
```
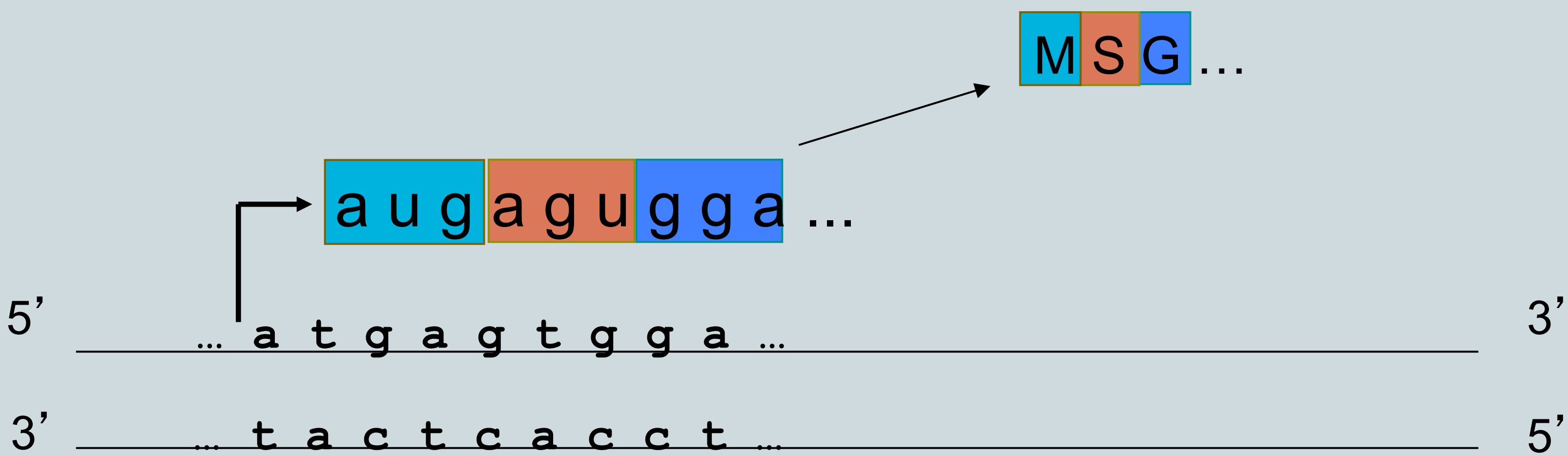

3-mers: codons

- We can extend the previous method to 3-mers
- k=3 is an important case in study of DNA sequences because of genetic code



3-mers in Escherichia coli genome

Word	Count	Observed	Expected	Word	Count	Observed	Expected
AAA	108924	**0.02348**	**0.01492**	CAA	76614	0.01651	0.01541
AAC	82582	0.01780	0.01541	CAC	66751	0.01439	0.01591
AAG	63369	0.01366	0.01537	CAG	104799	0.02259	0.01588
AAT	82995	0.01789	0.01490	CAT	76985	0.01659	0.01539
ACA	58637	0.01264	0.01541	CCA	86436	0.01863	0.01591
ACC	74897	0.01614	0.01591	CCC	47775	0.01030	0.01643
ACG	73263	0.01579	0.01588	CCG	87036	0.01876	0.01640
ACT	49865	0.01075	0.01539	CCT	50426	0.01087	0.01589
AGA	56621	0.01220	0.01537	CGA	70938	0.01529	0.01588
AGC	80860	0.01743	0.01588	CGC	115695	**0.02494**	**0.01640**
AGG	50624	0.01091	0.01584	CGG	86877	0.01872	0.01636
AGT	49772	0.01073	0.01536	CGT	73160	0.01577	0.01586
ATA	63697	0.01373	0.01490	CTA	26764	**0.00577**	**0.01539**
ATC	86486	0.01864	0.01539	CTC	42733	0.00921	0.01589
ATG	76238	0.01643	0.01536	CTG	102909	0.02218	0.01586
ATT	83398	0.01797	0.01489	CTT	63655	0.01372	0.01537

3-mers in Escherichia coli genome

Word	Count	Observed	Expected	Word	Count	Observed	Expected
GAA	83494	0.01800	0.01537	TAA	68838	0.01484	0.01490
GAC	54737	0.01180	0.01588	TAC	52592	0.01134	0.01539
GAG	42465	0.00915	0.01584	*TAG*	27243	**0.00587**	**0.01536**
GAT	86551	0.01865	0.01536	TAT	63288	0.01364	0.01489
GCA	96028	0.02070	0.01588	TCA	84048	0.01812	0.01539
GCC	92973	0.02004	0.01640	TCC	56028	0.01208	0.01589
GCG	114632	**0.02471**	**0.01636**	TCG	71739	0.01546	0.01586
GCT	80298	0.01731	0.01586	TCT	55472	0.01196	0.01537
GGA	56197	0.01211	0.01584	TGA	83491	0.01800	0.01536
GGC	92144	0.01986	0.01636	TGC	95232	0.02053	0.01586
GGG	47495	0.01024	0.01632	TGG	85141	0.01835	0.01582
GGT	74301	0.01601	0.01582	TGT	58375	0.01258	0.01534
GTA	52672	0.01135	0.01536	TTA	68828	0.01483	0.01489
GTC	54221	0.01169	0.01586	TTC	83848	0.01807	0.01537
GTG	66117	0.01425	0.01582	TTG	76975	0.01659	0.01534
GTT	82598	0.01780	0.01534	TTT	109831	0.02367	0.01487

2nd order Markov Chains

- Markov chains readily generalise to higher orders
- In 2nd order markov chain, position t depends on positions t-1 and t-2
- Transition matrix:

	A	C	G	T
AA				
AC				
AG				
AT				
CA				
...				

Codon Adaptation Index (CAI)

- Observation: cells prefer certain codons over others in highly expressed genes
  - Gene expression: DNA is transcribed into RNA (and possibly translated into protein)

Moderately expressed

Amino acid	Codon	Predicted	Gene class I	Gene class II
Phe	TTT	0.493	0.551	0.291
	TTC	0.507	0.449	0.709
Ala	GCT	0.246	0.145	0.275
	GCC	0.254	0.276	0.164
	GCA	0.246	0.196	0.240
	GCG	0.254	0.382	0.323
Asn	AAT	0.493	0.409	0.172
	AAC	0.507	0.591	0.828

Highly expressed

Codon frequencies for some genes in E. coli

Codon Adaptation Index (CAI)

- Consider an amino acid sequence $X = x_1x_2 \ldots x_n$
- Let p_k be the probability that codon k is used in highly expressed genes
- Let q_k be the highest probability that a codon coding for the same amino acid as codon k has
  - For example, if codon k is ”GCC”, the corresponding amino acid is Alanine (see genetic code table; also GCT, GCA, GCG code for Alanine)
  - Assume that $p_{GCC} = 0.164$, $p_{GCT} = 0.275$, $p_{GCA} = 0.240$, $p_{GCG} =$ **0.323**
  - Now $q_{GCC} = q_{GCT} = q_{GCA} = q_{GCG} =$ **0.323**

Codon Adaptation Index (CAI)

- CAI is defined as

$$\mathrm{CAI} = \left(\prod_{k=1}^{n} p_k / q_k\right)^{1/n}$$

- CAI can be given also in *log-odds* form:

$$\log(\mathrm{CAI}) = (1/n) \sum_{k=1}^{n} \log(p_k / q_k)$$

CAI: example with an E. coli gene

q_k

$\boxed{p_k}$

M	A	L	T	K	A	E	M	S	E	Y	L	…
ATG	GCG	CTT	ACA	AAA	GCT	GAA	ATG	TCA	GAA	TAT	CTG	
1.00	**0.47**	0.02	0.45	**0.80**	**0.47**	**0.79**	**1.00**	**0.43**	**0.79**	0.19	0.02	
	0.06	0.02	**0.47**	0.20	0.06	0.21		0.32	0.21	**0.81**	0.02	
	0.28	0.04	0.04		0.28			0.03			0.04	
	0.20	0.03	0.05		0.20			0.01			0.03	
		0.01						0.04			0.01	
		0.89						0.18			**0.89**	
ATG	**GCT**	TTA	ACT	**AAA**	**GCT**	**GAA**	**ATG**	**TCT**	**GAA**	TAT	TTA	
	GCC	TTG	**ACC**	AAG	GCC	GAG		TCC	GAG	**TAC**	TTG	
	GCA	CTT	ACA		GCA			TCA			CTT	
	GCG	CTC	ACG		GCG			TCG			CTC	
		CTA						AGT			CTA	
		CTG						AGC			**CTG**	

$$\left(\frac{1.00 \; 0.20 \; 0.04 \; 0.04 \; 0.80 \; 0.47 \; 0.79 \; 1.00 \; 0.03 \; 0.79 \; 0.19 \; 0.89 \ldots}{1.00 \; 0.47 \; 0.89 \; 0.47 \; 0.80 \; 0.47 \; 0.79 \; 1.00 \; 0.43 \; 0.79 \; 0.81 \; 0.89} \right)^{1/n}$$

CAI: properties

- CAI = 1.0 : each codon was the most frequently used codon in highly expressed genes
- Log-odds used to avoid numerical problems
  - What happens if you multiply many values <1.0 together?
- In a sample of E.coli genes, CAI ranged from 0.2 to 0.85
- CAI correlates with mRNA levels: can be used to predict high expression levels

Biological words: summary

- Simple 1-, 2- and 3-mer models can describe interesting properties of DNA sequences
  - GC skew can identify DNA replication origins
  - It can also reveal *genome rearrangement* events and *lateral transfer* of DNA
  - GC content can be used to locate genes: human genes are comparably GC-rich
  - CAI predicts high gene expression levels

Biological words: summary

- k=3 models can help to identify correct *reading frames*
  - Reading frame starts from a start codon and stops in a stop codon
  - Consider what happens to translation when a single extra base is introduced in a reading frame
- Also word models for k > 3 have their uses